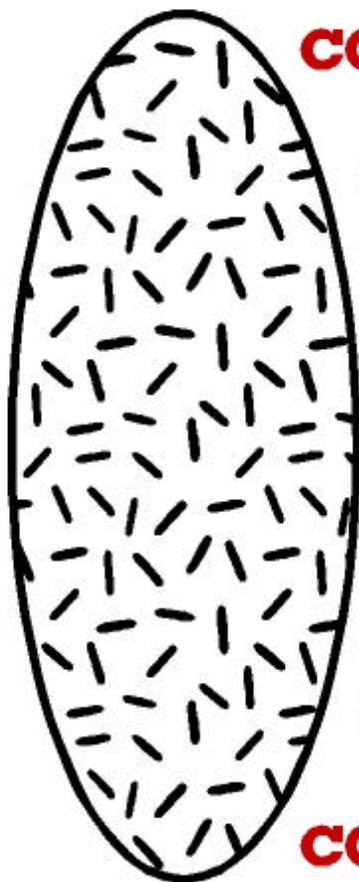


U.S. DEPARTMENT OF ENERGY

Natural and Accelerated Bioremediation Program

Workshop Report



**COMBINED
CHEMICAL AND
MICROBIOLOGICAL
APPROACHES TO
REMEDATING METAL
AND RADIONUCLIDE
CONTAMINANTS**

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October 28-29, 1999, Dulles Airport Marriott, Virginia

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Introduction

A two-day workshop titled “**Combined Chemical and Microbiological Approaches to Remediating Metal and Radionuclide Contaminants**” was held on October 28-29,1999 in Washington, DC (Dulles Airport Marriott Hotel) under the auspices of the **Natural and Accelerated Bioremediation Research Program (NABIR)** of the Office of Biological and Environmental Research, U.S. Department of Energy. A major objective was to bring together chemists and microbiologists to identify important research areas and applications for *in situ* bioremediation of metals and radionuclides through a synthesis of chemical and microbiological approaches. The issues were addressed in two broad sessions involving a series of **oral presentations** and topically organized **discussions by break-out groups**.

About This Report

This report summarizes the key findings of the breakout groups. Appendices A, B and C, respectively, contain the abstracts of the oral presentations, the meeting’s agenda, and the list of participants.

Acknowledgements

We would like to thank Ronald L. Crawford, Raymond E. Wildung, and John C. Houghton for their enormous input during the planning of the workshop. We are grateful to Claire Lamberti for her help in the organization of the workshop. We are particularly thankful to all the speakers and participants for their enthusiasm, support, and sharing their ideas freely during discussions. We acknowledge the NABIR program, Environmental Sciences Division, U.S. Department of Energy for financial support.

Background and Workshop Objectives

The contamination of subsurface sediments, soils, and groundwater by toxic heavy metals and radionuclides through the disposal of wastes and by-products from industrial- and defense-related activities is not only a growing problem worldwide, but also a major issue that raises serious and urgent concerns at some Department of Energy (DOE) sites. The primary goal of the DOE's NABIR program is to develop a fundamental scientific basis for intrinsic and accelerated *in situ* bioremediation of metals and radionuclides at these sites. Remediating metal contamination is particularly challenging because they cannot be chemically degraded, unlike most organic pollutants. Therefore, developing the technologies to remediate metals and radionuclides requires innovative solutions combining multifaceted approaches. The DOE's main purpose in sponsoring this workshop was to bring together environmental chemists, microbiologists and engineers to explore the directions of, and to initiate interest in, future interdisciplinary technologies, particularly those combining chemistry and microbiology. In general, metal-remediation technologies are based on two principles: mobilization and stabilization. Thus, a major emphasis was placed on these two themes dealing with different aspects of combining chemical and microbiological solutions.

Findings of Breakout Groups

Discussion Topics

The discussions were organized under four topics assigned to different groups:

- 1) *In Situ* Immobilization — Maximizing the Removal of Metals and Radionuclide Contaminants from the Aqueous Phase and Stabilizing Them
- 2) *In Situ* Immobilization — Minimizing Remobilization of Contaminants
- 3) Mobilization to Remove Contaminants from the Subsurface
- 4) Moving Basic Research on Combined Chemical and Biological Approaches to the Field

The groups were asked to identify gaps in knowledge, novel combinations of chemical and biological approaches and the potential to adapt *ex situ* treatments for *in situ* approaches.

***In Situ* Immobilization — Maximizing the Removal of Metals and Radionuclide contaminants from the Aqueous Phase and Stabilizing Them**

Knowledge Gaps

Metal contaminants in subsurface systems can be immobilized with the appropriate chemical and/or biological methods by precipitating them in a stable form. For example, several transition metals can be precipitated as sulfides by delivering hydrogen sulfide to the environment. Currently, there is insufficient scientific and technical information to apply such approaches at contaminated sites. Major gaps include the following:

- Mechanisms of immobilization to better understand *in situ* reactions; the relative importance of abiotic vs. biotic processes in controlling speciation; the speciation of inorganics; and the effect of speciation on the stability of the mixed-metals phase
- Approaches for *in situ* delivery and control of immobilizing agents (e.g., H₂S) in subsurface vadose- and saturated-zones

- Microbe-metal interactions, including microscopic and submicroscopic characterization of the cellular microenvironment.
- Biogeochemical transformations of contaminants of importance to DOE including Pu and Tc; conversions in concentrated solutions (such as in source-plumes); *in situ* reaction kinetics; and the effects of advective- and diffusive-transport on biogeochemical reactions.
- Long-term studies to examine the physical, chemical and biological effects of treatments. For example, what are the effects of precipitation on the system after a biomineralization treatment?

Combined chem-bio approaches

The transport of immobilizing agents may be enhanced by coupled physical-chemical-biological approaches (e.g. electrokinetic, acoustic pulse, and surfactants). Coupled processes may be spatially or temporally separated for the long-term maintenance of optimal conditions for immobilization. The following are some areas for coupled microbiological and chemical processes for immobilizing metals requiring exploratory research:

- Innovative use of co-contaminants and/or nutrients
- Delivery of non-aqueous phase nutrients
- Biologically generated geochemical barriers

Ex situ approaches for in situ treatments

- Leaching using microbial metabolites for dissolution
- Mobilizing *in situ* contaminants for *ex situ* treatment
- *In situ* cementation or polymerization (formation of carbonates, oxides or a reduction in porosity, permeability)
- Super-critical fluid extraction
- Applying novel thermophiles/barophiles to transform metals

***In Situ* Immobilization — Minimizing Remobilization of Contaminants**

Knowledge Gaps

Information is critically lacking in three major areas: 1) biogeochemistry, 2) microbial ecology and 3) hydrology/modeling.

Biogeochemistry. Biogeochemical changes play a major role in controlling the long-term fate of immobilized contaminants. Further research concerning biogeochemical transformations should elucidate the following:

- Speciation of the immobilized contaminants, particularly radionuclides such as Tc, U and Pu. Currently, information is lacking about their valence, composition, surface area, protective coatings, spatial location (intracellular vs. extracellular) and geochemical/physical properties
- The nature and properties of redox buffering phases, including information about Fe, Mn mixed oxides, co-precipitates, carbonates, and organic materials
- Oxidation rates by various oxidants, including reactions with various contaminants and redox buffering-phases, competition between oxygen and other oxidants, and the kinetics and thermodynamics of these processes
- The fate of contaminants upon reoxidation, including their speciation and mechanisms and whether the contaminant is immobilized or solubilized
- Biogeochemistry on important radionuclides, including Tc and Pu

Microbial ecology. The effects of microbial ecology on long-term stability are not entirely understood. Important issues include the following:

- Ways to stimulate *in situ* communities to optimize the contaminants' stability. For example, approaches using various electron donors to stimulate the reduction of contaminants or the production of extracellular polymers (if plugging is acceptable)
- Recovery of communities after immobilization and how it affects their long-term stability
- Monitoring community structures to indicate oxidizing conditions since bacterial populations may be more sensitive than chemistry-based measurements of groundwater

- Case studies of natural metal concentrations and long-term immobilization; for example, studies to reveal the factors that maintain natural formations (e.g.Cigar Lake)

Hydrology/modeling. The following are some important gaps in our knowledge:

- Linking of models of biogeochemical processes with transport models; these would allow us to determine capture efficiency and contaminant concentrations to predict the long-term stability of contaminants, to separate the effects of treatment from natural attenuation and to document the maintenance of reducing/immobilizing conditions
- Impacts of subsurface heterogeneity on barrier emplacement, fractional reduction and “leaky spots”
- Impacts of non-uniform flow and spatial heterogeneity of sediments and microbes
- Consequences of anaerobic stimulation and reoxidation on hydrology and transport
- Effects of precipitates, colloids and biofilms on hydrology

Combined chem-bio approaches

Novel ways to combine chemical and biological approaches include the following:

- Scavenging up gradient oxygen from both the saturated- and unsaturated-zones (e.g., using biological or colloidal iron); electrical generation of hydrogen)
- Combining rapid chemical reduction with biological reduction for long-term maintenance without lowering the aquifer’s reducing capacity or destroying the natural microbial communities
- Mobilizing fast-desorbing contaminants (50-70%) then immobilizing them to better stabilize the “sequestered “fraction
- Preventing the flow from groundwater and the vadose zone moving into the immobilized zone by hydrologic means, , or concentrating groundwater flow into a funnel and gate to allow oxidation and capture of contaminants

Mobilization to Remove Contaminants from the Subsurface

Knowledge Gaps

In general, gaps in information are greater for radionuclides than for metals. Major ones occur in the following areas:

- Various biological and coupled biogeochemical processes controlling the mobilization of metals and radionuclides, as well as knowledge of speciation and the mechanisms involving such processes
- The influence of metal speciation on mobility; integrating speciation data into kinetic and thermodynamic modeling
- Novel approaches for effectively enhancing the mobilization of metals and radionuclides. Some important questions are 1) How do we monitor the ability of complexants to mobilize or immobilize contaminants *in situ*? 2) How can we enhance the selectivity, recovery, and longevity of these complexants? and 3) Are specially designed complexants feasible to optimize metal binding?
- Several problems occur when introducing organic complexants, nutrients or selective enhancers by pump and treat or electrokinetic processes into silts and clays to mobilize metals and radionuclides. For example, interaction of the injected material with the local bacterial population can affect its homogeneous penetration through biomass build-up that clogs the injection wells because, invariably, the bacteria proliferate faster than the injected material moves. Solutions to these kinetic limitations are needed, such as slower-reacting additives or timed-release mechanisms and programmed introduction of microbes,. Furthermore, a clear understanding of the microbial pathways at a given site is critical, including rates and mechanisms of transformations of the injected material, for effectively modeling and implementing the engineering design
- Information on the speciation of complexes of radionuclides at low concentrations, including their solubility and stability constants
- Knowledge of toxicity bioavailability as well and limits of acceptable environmental concentrations for several metals and radionuclides and their complexes
- Applying the laboratory data to the field, given its heterogeneity in geochemistry, microbiology, and flow. Field research is needed to quantify the recovery of mobilized metals, characterize heterogeneity and the post-treatment concentration (volume reduction) and to analyze the costs and benefits.

Combined chem-bio approaches

The following are some novel, combined approaches:

- The use of complexants and/or chelators combined with electrokinetics to alter the permeability of the subsurface, as well as the transport rates of bacteria, selective inducers and contaminants
- Mobilization of contaminants combined with barrier approaches or stabilization
- Control of redox conditions to mobilize contaminants
- Biochemical sensors to monitor the amount and speciation of metals and radionuclides

Ex situ approaches for in situ treatments

- The use of novel complexants to mobilize contaminants in pump-and-treat or electrokinetic-processes to extract and/or mobilize metals
- The use of specially developed bacteria and the adaptation of bioreactor control/optimization to *in situ* treatment, using the subsurface as a bioreactor.

Moving Basic Research on Combined Chemical and Biological Approaches to the Field

Gaps in Knowledge - Areas for Research

- Microbial adaptation/selection/evolution to metals, horizontal- and vertical-gene transfer; chemical/biological process dynamics (synergistic and long-term)
- Ways to accelerate the intrinsic kinetics of the system in the field, as well as knowledge of the limiting and uncertain mass-transfer processes
- Development of large-scale transport models, particularly for microbial ecology (models should add levels of complexity, but may also take advantage of scaling-down larger models)

- Knowledge of the speciation of metals and radionuclides in the field and their acceptable environmental levels.

NABIR should take advantage of existing knowledge, applying it to the following areas including: industrial chemical processes, chemical remediation (Fenton's reagent, KMnO_4), thermal remediation (gradients; thermophiles), natural analogue environments (geothermal environments and ore deposits), treatment of metals in wastewater, landfills (long-term studies, mixed waste); and metallurgical engineering (extraction chemistry) and barrier technologies (transport, funnel and gate, iron walls).

Practical Approaches for NABIR

- Take advantage of opportunities to scale-up work, such as those available in the field research centers
- Get exposed to field problems to understand the costs, milestones, baseline technologies, geology and hydrology that are part of the remedies
- Learn to interact with the people and with the regulators to solve problems
- Encourage interdisciplinary scientific teamwork
- Provide incentives to use new technologies
- Integrate research with several interrelated programs (including those of EM50, EM40 and EMSP)
- Take advantage of technologies in mining and related fields
- Ascertain transportability of any new technology from the laboratory to the field
- Provide access to web databases on existing field experiments

Appendix A

Abstracts of Oral Presentations (in order of presentation)

**Biotic and Abiotic Interactions Between Chlorinated Solvents, Microbial Metabolites,
and Metals: The example of *Pseudomonas stutzeri*, Strain KC**

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Pseudomonas stutzeri strain KC, while grown under iron limitation, produces and excretes a novel metal chelator we identified as pyridine-2,6-bis(thiocarboxylate), or PDTC. PDTC is unusual in that besides strongly complexing metals, it promotes the dehalogenation and mineralization of chlorinated solvents such as carbon tetrachloride (CT). We have cloned and characterized the genes required for the synthesis of PDTC, and are able to transfer these genes into other species of *Pseudomonas* such that they become able to synthesize and excrete PDTC. This leads to the possible use of *Pseudomonas stutzeri* strain KC (or other organisms expressing the PDTC genes) in mixed waste-contaminated environments to simultaneously address both organic and inorganic contaminant problems, and is an excellent example of a combined biotic and abiotic treatment strategy. However, the chemistry involved is quite complex. In our recent work we have begun looking at this complexity. PDTC and its metal complexes were synthesized using modifications of known methods. PDTC and its Fe, Ni, Zn, Co, Cu, Au, and Mn complexes were prepared and purified by crystallization. All compounds were > 95% pure. Divalent metals such as Cu and Zn formed 1:1 complexes with PDTC, while metals such as Fe, Co, and Mn formed 1:2 complexes. The structures of PDTC and its metal complexes were elucidated using electrospray negative ionization mass spectrometry (MS) of samples prepared in a water/methanol solution. For further confirmation of structures, daughter fragments were generated using collision-induced ionization with an argon gas-filled collision cell in an MS/MS spectrometer. The PDTC metal complexes then were examined for (a) their ability to dehalogenate CT, and (b) their binding affinities for different metals. These types of data will allow us to predict the usefulness of *in situ* biologically produced PDTC in mixed-waste environments for simultaneous degradation of chlorinated solvents and mobilization or immobilization of metals or radionuclides.

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Application of Biosurfactants in the Remediation of Metal and Organic Co-Contaminated Soils and Wastestreams

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The existence and fate of heavy metals in soil is of concern not only because of their potential impact on microbial communities which comprise a "healthy soil", but also because of the potential for groundwater contamination and hence, toxicological impact on human health. A major problem associated with remediation of subsurface systems contaminated by heavy metals is the difficulty in delivering the metals to the surface for subsequent treatment. Typically, "pump and treat" remediation does not attain cleanup within predicted timetables due to a "tailing" effect once initial high heavy metal concentrations have been flushed out. Further complicating remediation of metal-contaminated sites is the fact that a large proportion of such sites are co-contaminated with organics. Such co-contaminated sites are difficult to bioremediate due to the nature of the mixed contaminants. Specifically, the presence of a co-contaminating metal imposes increased stress on indigenous populations already impacted by organic contaminant stress. The complex nature of such contaminated sites may require that more than one treatment strategy be applied to achieve an acceptable level of remediation.

The research to be discussed concerns the application of microbially-produced surfactants (biosurfactants) to metal-contaminated sites. Results show that a rhamnolipid biosurfactant, when added at high concentration, has the potential to reduce the tailing effect associated with "pump and treat" and hence the amount of water pumped. Rhamnolipid is effective because it is selective for toxic metals such as lead and cadmium in the presence of naturally-occurring cations such as calcium or magnesium. Measured rhamnolipid-metal stability constants were also found in most cases to be similar or higher than conditional stability constants reported in the literature for metal complexation with sewage sludge, humic acids, and fulvic acids. Results from laboratory-scale saturated column experiments show that rhamnolipid can be very effective at removal of metals from soil, but its effectiveness is influenced by soil type. Therefore the soil characteristics that impact the effectiveness of this sorbing biosurfactant as a flushing agent were determined. Rhamnolipid sorption to soil is affected most by the amount and type of clay followed by the amount and type of metal oxide. Sorption is also affected by the rhamnolipid structure. The monorhamnolipid form is approximately 10 times better at metal complexation than the dirhamnolipid form, however, it sorbs more strongly. Therefore, a mixture of the two surfactants is more effective in metal removal from soil than the monorhamnolipid alone.

A second approach under investigation is the application of biosurfactants in lower concentration to co-contaminated sites. In this case, metal toxicity to biodegrading microbes is reduced allowing more rapid biodegradation of the organic contaminant. Results from a model solution system (phenanthrene and cadmium as co-contaminants) show that phenanthrene mineralization in the presence of cadmium can be stimulated to control levels by the addition of rhamnolipid. These results were then tested in a soil system to determine whether an indigenous soil population would behave similarly. In two soils mineralization of phenanthrene was driven to control levels by pulsed addition of rhamnolipid. Pulsed addition was required to maintain threshold

rhamnolipid levels because rhamnolipid is slowly degraded by the indigenous soil community. Taken together, these results help delineate the conditions under which rhamnolipids can be successfully applied as a remediation agent in metal-contaminated sites. This includes the mobilization and removal of metal contaminants from soil as well as surface waters, groundwater, and wastestreams.

The Role of Thiols and Sulfide Oxidation Intermediates in Mobilizing Metals

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A critical concept in understanding and predicting the mobility/immobility, toxicity, and bioavailability of toxic heavy metals in the environment is the speciation or chemical forms in which they exist. Several molecular-scale processes control the speciation of metal contaminants, including surface complexation or adsorption onto mineral surfaces, precipitation-dissolution reactions, redox transformation, and complexation by inorganic and organic ligands. Among the variety of ligands, carboxylated compounds are the dominant complexants of metals in the environment, and include natural organic molecules (mainly humic substances) and those originating from human activities (e.g., EDTA and citrate). Several compounds with sulfur-containing functional groups that can bind metals are present in anaerobic and sub-oxic environments. Hydrogen sulfide is a major sulfur compound in anaerobic environments; it sequesters heavy metals, precipitating them as metal sulfides. Under sub-oxic conditions, hydrogen sulfide is partially oxidized forming mainly thiosulfate and sulfate. Thiols also occur in reducing anaerobic environments either derived biochemically from organic detritus, or formed abiotically from the reactions of hydrogen sulfide with organic molecules.

Despite the importance of thiosulfate and thiols in anaerobic environments, little is known about their interactions with heavy metals. We systematically investigated the chemical reactions between cadmium, a highly toxic heavy metal, and a series of selected low-molecular-weight thiol compounds with varying structures and functional groups. A major goal was to better understand the effects of important environmental variables such as the chemical structures and concentration of thiols, and pH, on the mechanisms of formation and stability of the resulting complexes. A potentiometric method was used to study the reactions between Cd(II) and thiol compounds, with x-ray absorption spectroscopy to characterize the structures of the complexes.

Changes in pH dramatically affected the binding of thiols with Cd(II) binding declining with decreasing pH due to competition from protons. The stoichiometry of the binding reactions was affected by both pH and the ratio of Cd(II) to the thiol compound, while the structure and the precipitation/dissolution behavior of the complexes mainly depended on the molecular composition and the structure of the thiol compound. For example, three compounds, 3-mercaptopropionic acid, L-cysteine, and dithioerythritol, generated precipitates, but there were only soluble complexes for all other low-molecular-weight thiols examined. The 1:1 complex between 3-MPA and Cd(II) is expected to be present as a zwitterion in aqueous solution at neutral pH if only the -SH group binds with Cd(II); the carboxylic group should be ionized at this pH. The precipitation of this complex suggests that a charge-neutralized species probably emerged from the formation of a ring structure. Thus, it appears that although the carboxyl group alone had negligible binding to Cd(II), joint binding

with the sulfhydryl group may occur, yielding a stable six-membered ring structure. For all three thiol compounds which formed precipitates with Cd(II), a stable six-membered ring or seven-membered ring could be formed by the simultaneous binding of a single Cd(II) by the compound's two functional end groups.

While the structure of the thiols influenced the solubility of Cd-thiol complexes with pH, the 1:2 complex with thiosulfate was completely soluble over the 4-8 pH range. A new bacterium, *Klebsiella planticola* strain Cd-1, that we isolated from salt-marsh sediments in Shelter Island, New York grew well in the presence of high (mM levels) levels of Cd(II)-thiosulfate complex and transformed it to cadmium sulfide. We compared the ability of Cd-1 with that of several metal-resistant, metal-transforming facultative anaerobes to grow in Cd(II)-containing solutions amended with thiosulfate. Our results clearly showed that Cd-1 is superior in anaerobically transforming Cd(II) to CdS, particularly when present as complexes of thiosulfate. Because thiosulfate forms highly soluble complexes with cadmium, it can be used to mobilize and extract cadmium using a "pump and treat" process for *in situ* remediation of contaminated soils and sediments. After extraction, the metal-containing water can be treated with a bacterium, such as Cd-1, to bioprecipitate cadmium as CdS. The possible use of thiosulfate complexation in a pump-and-treat application, followed by bacterially induced precipitation as CdS from the extracted solution illustrates a remediation application combining chemical and microbiological approaches.

The Role of Chelates in Metal and Radionuclide Mobilization

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Multidentate synthetic chelating agents such as ethylenediaminetetraacetate (EDTA) and nitrilotriacetate (NTA) were used to decontaminate nuclear reactors and other materials and for processing nuclear waste, because they form stable water-soluble complexes with a wide range of radionuclides and metals. The co-disposal of synthetic chelating agents with radionuclides at U.S. Department of Energy (DOE) sites has enhanced the transport of radionuclides and metals in groundwater. The enhanced mobility of cationic radionuclides in groundwater is presumably because of altered adsorption and retardation of the radionuclides because they are chelated. The synthetic chelation agents could also be used to mobilize radionuclides and metals disposed to sediments and currently immobile. Processes of importance to consider for technology deployment include the sorption/desorption of the chelate and various chelate-metal complexes to various sorbents. Factors to consider include sorbent type, pH, concentrations of metals and chelate, form of the contaminant in the sediments, competing ions, and redox. It is also important to understand and be able to model the thermodynamics and/or kinetics of reactions important for the processes of sorption, aqueous speciation, dissociation of the metal-chelate complex, oxidation/reduction of metals, dissolution of sorbents by the chelate, biodegradation of the chelate, and transport in the sediment. These coupled interactions of geochemistry, microbiology, and hydrology are important to understand in designing strategies for chelate assisted radionuclide mobilization.

Electrokinetic Remediation of Heavy Metals and Radionuclides

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Electrokinetic soil processing comprises a group of emerging technologies with prospects for the extraction or immobilization of many heavy metals and radionuclides, or the extraction of, or enhanced bio degradation of toxic organic contaminants. Electrokinetic remediation is a technique where electrodes are placed in an open or closed flow arrangement across the soil mass to employ a very low DC electrical current density to transport the species under coupled and/or uncoupled conduction phenomena. These processes result in physicochemical and hydrological changes in the soil matrix. Generally, externally supplied fluid or groundwater, acts as the conductive medium. The transport of the species under electrical fields is influenced by the prevailing electrolysis reactions at the electrodes. Although the fundamental principles of the electrokinetic processes are well understood, the complexities of real site conditions and particularly the chemical and geochemical factors, more frequently than not play a role in selection of the optimal extraction methodology. It is recommended that pre-characterization of site samples be made in laboratory bench-feasibility tests including sequential extraction, buffer capacity, conductance, etc.. Computer programs have been developed that are able to model the transport behavior and the local chemistry (adsorption, precipitation, complexation effects, etc.) for multiple metal species and in principle these can aid in method design. Modeling has also been developed to aid in site design of wells and electrodes.

The technology has been studied mainly for the remediation of inorganic species; however, it is possible to employ electrokinetics in bioremediation to engender an effective level of injected nutrients, electron acceptors/donors, microbes or other process additives in a soil matrix. The process additives can be injected into the system at the electrodes by the electrolysis reactions or by cycling processing fluids in the electrode wells. However, unless the rate of injection of an additive is made compatible with its rate of consumption, column results with TCE contaminant and a theoretical model reveal that homogeneous penetration of additive is not achieved. It is cautioned that knowledge of the rate of degradation of a carbon source enhancer (or additive) is critical for engineering its homogeneous injection, whether by hydraulic or electrokinetic methods. As more data from full-field remediations become available, these electrokinetic technologies may be the methods of choice for in situ remediation of many sites including those with mixed (inorganic/organic) pollutants.

Biogeochemical Stabilization of Metals and Radionuclides by Sorption at Mineral-Water Interfaces

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Sorption processes localize solutes at the mineral-water interface and are responsible for attenuating the rate of subsurface contaminant migration and for driving heterogeneous reactions on particle surfaces. Whether biologic or abiotic, sorption processes are directly or indirectly involved in any in-situ subsurface remediation process. Sorption is a general term that encompasses multiple reaction suites including adsorption, ion exchange, and surface precipitation. While these processes have been well studied, they are complex and the focus of much current geochemical research. This presentation will focus on sorption processes associated with bacterial iron reduction, emphasizing reactions that impact the solubility of metal and radionuclide contaminants of DOE concern. Bacterial Fe(III) oxide reduction is highlighted because of its important impact on multivalent metal speciation, and its prominent role in both mobilizing and immobilizing inorganic contaminants. Specific examples from recent research will be provided on the coupling of sorption processes with bacterial iron reduction including: 1.) reductive immobilization by redox reaction with sorbed Fe(II) [e.g., Tc(VII) and U(VI)], 2.) adsorption and coprecipitation reactions controlling mobilization/immobilization of oxide-bound contaminants (Co, Ni) during bioreduction, 3.) role of biogenic Fe(II) on electron acceptor properties and bioavailability, and 4.) influence of sorbed metals (Ni, Co) on ferrihydrite crystallization and bioreducibility. The presentation will conclude with discussion of a conceptual model for how sorption processes couple with bacterial iron reduction to control contaminant dynamics both in the natural and engineered setting.

Competing Biological and Geochemical Processes in Metal and Radionuclide Reduction

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Reductive processes, whether abiotic or biotic, may serve to stabilize toxic heavy metals and radionuclides such as chromium and uranium. Direct enzymatic reduction of chromate and uranyl during bacterial respiration is highly desirable as it should lead to reduced products having limited solubilities. Unfortunately, competing electron acceptors may impede the reduction of a target phase.

Conversely, in some cases metabolic products formed during the reduction of alternate electron acceptors may lead to a chemical pathway for the reduction of the target element. Here we discuss and demonstrate the influences of competing electron acceptors on the reduction of uranyl and chromate by dissimilatory iron reducing bacteria (DIRB).

Ferric iron is ubiquitous in nature and in many suboxic soil, sediment and subsurface environments it is likely to be the most abundant terminal electron acceptor for microbial respiration. We investigated the microbially mediated, indirect reduction of chromate by *Shewanella alga* strain

BrY, by injecting a continuous stream of chromate into a suspension of BrY and hydrous ferric oxide minerals. In such systems, Fe(II) produced as a result of microbial respiration serves as a catalyst (or an electron shuttle), reducing Cr(VI) and thus being reoxidized. This cycling of iron suggests that a limited quantity of iron can potentially cycle indefinitely serving to stabilize chromate *in situ*. Furthermore, chromium(III) resulting from the process forms a solid-phase of very limited solubility.

In contrast to the reductive promoting effect noted for ferric iron with chromate, it may potentially serve to restrict the enzymatic reduction of uranyl. Microbial dissimilatory reduction of the highly soluble uranyl ion (U^{VI}) to relatively insoluble uraninite (UO_2) offers the potential for *in situ* stabilization. Uranium stabilization by this processes is dependent on uranyl being used as the terminal electron acceptor (TEA) in microbial respiration. We therefore examined the reduction of uranyl by BrY in the presence (and absence) of environmentally relevant iron hydrous oxides. When cell suspensions of BrY were added to uranyl acetate, uranyl was rapidly removed from solution. Similarly, uranyl adsorbed on goethite underwent dramatic reduction (>90%) with active BrY cells. In contrast, limited reduction was noted when ferrihydrite or amorphous ferric hydroxides were present in suspension. Our results demonstrate that alternate or competing electron acceptors present in soils may modify the reduction of toxic elements; they may either promote or retard the reduction of the target element. In either case, one must consider the specific site geochemistry when evaluating the potential for *in situ* reduction of uranyl or chromate.

Microbial Influence on Mobility of Metals and Potential for Bioremediation of Solid and Liquid Wastes

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Microorganisms are intimately involved in the biogeochemical cycling of metals and metalloids. Certain microbial processes solubilize metals thereby increasing their mobility, which may increase bioavailability and potential toxicity, whereas other processes result in immobilization and reduce bioavailability. The relative balance between mobilization and immobilization varies depending on the organisms involved, their environment and associated physico-chemical conditions. These processes may also be exploited for the treatment of contaminated solid and liquid wastes [1,2]. Metal mobilization can arise from autotrophic and heterotrophic leaching, chelation by metabolites and siderophores, and methylation which can result in volatilization. Immobilization can result from sorption to cell components or exopolymers, transport and intracellular sequestration or precipitation as organic and inorganic compounds, e.g. oxalates in fungi, and sulphides in sulphate-reducing bacteria. In addition, biologically-mediated reduction of higher-valency species may effect mobilization, e.g. Mn(IV) to Mn(II), or immobilization, e.g. Cr(VI) to Cr(III). In the context of bioremediation, solubilization of metal contaminants provides a means of removal from solid matrices such as soils, sediments, dumps and other solid industrial wastes. Alternatively, immobilization processes may enable metals to be transformed *in situ* and are particularly applicable to removing metals from aqueous solution.

This contribution will detail selected microbiological processes which are of significance in determining metal mobility and which have actual or potential applications in bioremediation of metal pollution. These include autotrophic and heterotrophic leaching mechanisms, sulphate reduction, and metal sulphide precipitation. A particular example is the application of microbially-catalyzed reactions which occur in the natural sulphur cycle in an integrated microbiological process to remove toxic metals from soils [3]. Here, bioleaching using sulphur-oxidizing bacteria was followed by precipitation of leachate metals as insoluble sulphides by sulphate-reducing bacteria. Metal contaminants including Cd, Co, Cr, Cu, Mn, Ni and Zn were efficiently leached from an artificially-contaminated soil: Mn, Ni and Zn were the only target elements which were significantly leached from soil minerals. An industrially-contaminated soil was also treated and approximately 70% of the main toxic metals present, Cu, Ni and Mn, were removed after 175 days. The leachate was stripped of metals using an anaerobic bioreactor containing a mixed culture of sulphate-reducing bacteria. More than 98% of the metals were removed from solution as solid metal sulphides with the exception of Mn, Ni and Pb where 80-90% were removed. Heterotrophic leaching by organic acid producing fungi provides a means of metal solubilization from insoluble metal compounds including solid wastes and minerals. The formation of mobile metal-organic acid anion complexes can result in transfer between different environmental compartments, while insoluble metal oxalate formation results in immobilization and may accompany heterotrophic leaching [4]. For example, we have demonstrated fungal solubilization of pyromorphite ($\text{Pb}_5(\text{PO}_4)_3\text{Cl}$), the most stable lead mineral found in soil under a range of physical and chemical conditions and itself advocated as a bioremediation process where Pb-contaminated soils receive phosphate amendments. The pyromorphite was additionally transformed to lead oxalate dihydrate, the first demonstration of the biogenic formation of this mineral [5]. This study emphasises the importance of considering microbial processes and activities in proposed remedial treatments for soils.

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Factors Influencing Stability of Metals and Radionuclides Immobilized under Sulfate Reducing Conditions

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Biologically-mediated in-situ immobilization is being considered as a means to significantly decrease transport rates of metallic contaminants. The creation of sulfate-reducing conditions to immobilize metals has potential use at many contaminated sites because of the large number of metals (e.g. Hg, Pb, Cd, Cu, Ni, Zn) that form stable sulfide compounds. In addition, effective reduction and subsequent precipitation of uranium (U) and chromium (Cr) under these conditions has been shown. Biological immobilization of metals in the saturated zone also has the potential to be combined with a chemical technology for vadose zone treatment for a more unified approach to subsurface remediation. However, as with other possible treatments, the long-term stability of the immobilized metals and radionuclides, and the factors that affect the immobilization/remobilization process must be quantified to determine whether the treatment can produce an acceptable endpoint.

Methods have been developed to test the hypothesis that the rate of metal remobilization will largely depend on the following factors: aquifer reductive capacity (generated in the form of iron sulfides), distribution and morphology of contaminant precipitates on mineral surfaces, and accessibility of contaminant precipitates to dissolved oxygen.

Specially designed flat-plate flow cells that contain pure and mixed redox-sensitive and insensitive minerals allow us to determine the spatial relationships between SRB colony location and activity, and the location and type of contaminant and sulfide mineral deposits on a hematite, or other aquifer-relevant surface.

A green fluorescent protein (GFP), from the jellyfish *Aequorea victoria*, was introduced into *Desulfovibrio desulfuricans* for direct observation of SRB on hematite using fluorescence microscopy. Unlike fluorescent stains, the GFP allows non-destructive, real-time observation of active SRB colonization and biofilm growth in both pure and mixed cultures. After the mineral coupon surface has been exposed to SRB activity, it is rinsed and examined using X-ray photoelectron spectroscopy (XPS) for surface characterization. XPS data indicate the formation of reduced iron sulfide on the hematite surface and also the presence of numerous sulfur species including S^{2-} , S_2^{2-} , S_n^{2-} , SO_3^{2-} , and SO_4^{2-} . The iron sulfide deposit on (001) hematite was identified as pyrrhotite, which is a stable, iron-deficient sulfide (FeS_{1-x}). These results are to be followed up with grazing angle XRD to determine the crystalline structure of the resulting iron precipitate. The formation of pyrrhotite is in contrast to many reports of iron sulfide formation in suspended SRB culture, where with no solid phase present, amorphous mixtures of the metastable mackinawite and greigite are typically precipitated. As a direct measure of SRB activity in mixed culture biofilms, microelectrode measurements of colony-scale H_2S profiles on inert surfaces will also be presented. Work is currently in progress to measure H_2S profiles on hematite surfaces to quantify the influence of SRB on microscale H_2S gradients in the presence of redox-sensitive minerals (e.g. hematite).

Zero-Valent Iron for In Situ Remediation of Selected Heavy Metals and Radionuclides in Permeable Reactive Walls

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The application of zero-valent iron for in situ remediation of contaminated groundwater in permeable reactive walls will be discussed. Emphasis will be placed on applications for toxic metals and radionuclides. Reactions of zero-valent iron with contaminants and major groundwater constituents will be included. The discussion will include what is currently known regarding reaction mechanisms and will include aspects from geochemistry, corrosion science and microbiology.

Fielding Combined Chemical and Biological Approaches

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Selenium mobilization from mining operation waste rock in the Western US Phosphate Region has led to environmental contamination concerns. Observations of water Se levels that significantly exceed aquatic biota criteria present a control challenge near the vast waste rock impoundments. Historical mining operations have resulted in large, waste rock canyon fills of approximately 1 km in length and 150 m in height near the 95 active or inactive mine sites. Infiltration of precipitation and snowmelt mobilizes large amounts of leached selenium in the canyon drainages with the potential for significant environmental impact. Other metals observed in the waste rock leachate include Cd, V, Ni and Zn.

In our prior work on the characterization of the reaction pathway, we observed Se release from seleno-pyrite and Se(0) in the middle waste shales of the waste rock. This oxidative pathway is facilitated by the presence of phosphate, which successfully competes for iron thereby aiding in the release of selenite. The mobilized selenite is further oxidized to selenate. Using laboratory batch reactors, we apply a combined abiotic and biotic approach for *in situ* stabilization using waste rock soil amendments. Several of the amendments included polyanionic biopolymers and iron. Amendments examined include scrap iron granules, colloidal iron, potato processing waste, potato starch, cheese whey and Fe(III)-thermal polyaspartic acid (tpA), with and without inoculation with sulfate reducing bacteria, *Desulfatamaculum orientis* and *Desulfovibrio desulfuricans*. We find evidence for stimulation of sulfate reducing bacteria (SRB) populations and Se immobilizing chemistry in the treated, water-saturated, waste rock soils. Water levels approaching 3 mg/L total Se are significantly reduced in all cases of treatment (>90%) with some treatments achieving 2 µg/L total Se levels in 14 days.

In this work, we present the results of two field studies of a combined abiotic/biotic zone amendment approach. In a sub-surface zone amendment approach, we placed 4 treatment cells in a 150 m (l) x 15 m (w) x 6 m (d) excavation. We developed a sub-surface sampling assembly (SSA,

n=4 in each cell) that consisted of a collection pan lysimeter and suction lysimeter at -3 m, connected to 250 L totalizing flow, collection barrel. All of the sampling areas in the SSA were accessible by wellhead or by sampling tubing. 0.7 m of homogenized waste rock soil was laid directly over the sampling zone. This zone was further covered with 5 cm iron metal granules (cell 2), 5 cm of iron granules plus 0.7 m of potato waste amended soil (cell 3), or 0.7 m of potato waste amended soil (cell 4). Each of the cells was then covered to ground level with homogenized waste rock soil. In an additional study, surface treatments of 30 m x 10 m plots with suction lysimeters (n=3) at -1 m were examined. The treatments in this study were iron metal granules and cheese whey. The results of the subsurface study indicate the horizontal amended zone at - 3 m reduces Se in the subsurface water by >85%. The results of the surface applied cheese whey indicate a 99% reduction of Se in the shallow subsurface (-1 m) water collection versus control.

Appendix B

Workshop Agenda

DOE NABIR Biotransformation/Biogeochemistry Workshop

Combined Chemical and Microbiological Approaches to Remediating Metal and Radionuclide Contaminants

Purpose:

The contamination of subsurface sediments, ground water, and soils by toxic heavy metals and radionuclides through the disposal of wastes and byproducts from industrial and defense-related activities is not only a growing problem worldwide, but also raises serious and urgent concerns at some Department of Energy (DOE) sites. Developing technologies to remediate metals and radionuclides requires interdisciplinary research. The DOE's purpose in sponsoring this workshop is to bring together environmental chemists, microbiologists, and engineers to explore the directions of, and to initiate interest in, future research based on such an interdisciplinary footing. In general, metal-remediation strategies are based on two principles (1) mobilization, and (2) stabilization. Most talks address these two themes, dealing with different aspects of chemical and microbiological solutions. Alternate approaches, such as electro-kinetic remediation, also will be discussed. We expect that this workshop will help to identify crucial research topics and approaches that will lead to future multidisciplinary research, particularly those combining chemical and biological approaches.

AGENDA

Thursday, October 28, 1999

8:10-8:20 Welcome and Introduction - Anna Palmisano and Murthy Vairavamurthy

Session I: Remediation of Metals and Radionuclides

8:20-8:30 **Discussion Leader:** James Fredrickson (Pacific Northwest National Laboratory)

8:30-9:00 Ronald L. Crawford (University of Idaho) - Mechanisms of metal and radionuclide immobilization/mobilization in subsurface sediments: an overview

9:00-9:30 Jeffrey S. Walker (DOE Office of Technology Systems, EM-53) - The status of bioremediation technologies involved in DOE's site cleanups

Session II: Mobilization of Metals in Subsurface Systems: The Influence of Chemical and Biological Mechanisms

- 9:30-9:40 **Discussion Leader:** Thomas Kieft (New Mexico Technology)
- 9:40-10:10 Raina Maier (University of Arizona) - Application of biosurfactants in the remediation of metal and organic-contaminated soils and wastestreams
- 10:10-10:30 Break
- 10:30-11:00 Murthy Vairavamurthy (Brookhaven National Laboratory) - The role of thiols and sulfide oxidation intermediates in metal mobilization
- 11:00-11:30 Harvey Bolton (Pacific Northwest National Laboratory) - The role chelators in metal and radionuclide mobilization
- 11:30-12:00 Robert J. Gale (Louisiana State University) - Electro-kinetic remediation of heavy metals and radionuclides
- 12:00-13:00 Lunch

Session III: Stabilization of Metals and Radionuclides in Subsurface Environments

- 13:00-13:10 **Discussion Leader:** Mary Neu (Los Alamos National Laboratory)
- 13:10-13:40 John Zachara (Pacific Northwest National Laboratory) - Biogeochemical stabilization of metals and radionuclides by sorption at mineral-water interfaces
- 13:40-14:10 Scott Fendorf (Stanford University) - Competing biological and geochemical processes in metal and radionuclide reduction
- 14:10-14:40 G.M. Gadd (University of Dundee, Scotland) - Microbial influence on metal mobility and potential for bioremediation of solid and liquid wastes
- 14:40-15:10 Brent Peyton (Washington State University) - Factors Influencing stability of metals and radionuclides immobilized under sulfate reducing conditions.
- 15:10-15:30 Break

Session IV. Barrier approaches to chemical/biological in situ treatment

- 15:30-15:40 **Discussion Leader:** Terry Hazen (Lawrence Berkeley National Laboratory)
- 15:40-16:10 Kirk J. Cantrell (Pacific Northwest National Laboratory) - Zero-valent iron for *in situ* remediation of selected radionuclides and heavy metals in permeable reactive walls
- 16:10-16:40 Greg Moller (University of Idaho) - Fielding combined chemical/biological approaches
- 16:40-16:50 Break
- 16:50-17:00 **Introduction to breakout group sessions** - Anna Palmisano
(Four discussion topics are planned: (1) In situ Immobilization I. Maximizing the Removal of Metals and Radionuclides from Aqueous Media, (2) *In situ* Immobilization II. Minimizing Remobilization of Stabilized Metals and Radionuclides, (3) Mobilization to Remove Metals and Radionuclides, and (4) Moving Basic Research on Combined Chemical and Biological Approaches to the Field. **A representative from each group will present the results of the discussions to all participants.** Key results will be incorporated in a short written summary of the meeting to be prepared by Vairavamurthy.)
- 17:00-18:00 Breakout groups convene (see the attachment for details of group assignments)

Friday, October 29, 1999

- 8:30-10:00 Breakout groups reconvene
- 10:00-11:00 Breakout groups report to all participants
- 11:00-12:00 Workshop wrap up - Anna Palmisano/ John Houghton

Breakout Group Discussion Topics

Each group should address the following topics:

- 1) How limited is our current knowledge of chemical/biological approaches? Where are the gaps in our knowledge?
- 2) What are novel ways to combine different approaches that might lead to new *in situ* bioremediation strategies or technologies?
- 3) Are there *ex situ* approaches that could be adapted to *in situ* bioremediation, and what kinds of research would be needed?

Group I. *In situ Immobilization I. Maximizing the Removal of Metals and Radionuclides from Aqueous Media*

Jim Frederickson (lead, rapporteur)
Mary Neu (co-lead)
Bill Burgos
Scott Fendorf
Geoffrey Gadd
John Heidelberg
Ken Kemner
Jim Petersen

Group II. *In situ Immobilization II. Minimizing Remobilization of Stabilized Metals and Radionuclides*

John Zachara (lead)
Dave White (co-lead)
Brent Peyton (rapporteur)
Winston Chen
Brian Dempsey
Yuri Gorby
Eric Hacherl
Jani Ingram
Derek Lovley

Group III. *Mobilization to Remove Metals and Radionuclides*

Raina Maier (lead)
Scott Brooks (co-lead)
Harvey Bolton (rapporteur)
Robert Gale
Baohua Gu
Jon Lloyd
Sarah MacNaughton
Flynn Picardal
David Scala

Group IV. *Moving Basic Research on Combined Chemical and Biological Approaches to the Field*

Terry Hazen (lead, rapporteur)
Greg Moller (co-lead)
David Balkwill
Kirk Cantrell
Tom Kieft
David Kosson
Andrzej Paszczynski
David Watson
Creighton Wirick

Appendix C

List of Participants

DOE NABIR Workshop - List of Participants

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